CHARACTERIZATION OF HUMAN CYTOCHROME P450 ENZYMES INVOLVED IN THE METABOLISM OF THE PIPERIDINE-TYPE PHENOTHIAZINE NEUROLEPTIC THIORIDAZINE

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ABSTRACT:

The aim of the present study was to identify human cytochrome P450 enzymes (P450s) involved in mono-2-, di-2-, and 5-sulfoxidation, and N-demethylation of the piperidine-type phenothiazine neuroleptic thioridazine in the human liver. The experiments were performed in vitro using cDNA-expressed human P450s (Superomics 1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, and 3A4), liver microsomes from different donors, and P450-selective inhibitors. The results indicate that CYP1A2 and CYP3A4 are the main enzymes responsible for 5-sulfoxidation and N-demethylation (34–52%), whereas CYP2D6 is the basic enzyme that catalyzes mono-2- and di-2-sulfoxidation of thioridazine in human liver (49 and 64%, respectively). Besides CYP2D6, CYP3A4 contributes to a noticeable degree to thioridazine mono-2-sulfonation (22%). Therefore, the thioridazine metabolism seems to be of importance.

Thioridazine, a prototypic agent for phenothiazine neuroleptics of the piperidine type, is a mild neuroleptic which acts on positive and negative symptoms of schizophrenia, displaying sedative and antidepresant effects. Because of its psychotropic profile, thioridazine is suitable for combination with antidepressants in the therapy of many psychiatric disorders (psychotic depression, “treatment-resistant” depression, depression in the course of schizophrenia, schizoaffective psychosis). However, the main side effects of thioridazine are related to cardiac muscle conduction and anticholinergic activity. Among neuroleptic drugs, thioridazine produces the most distinct ECG abnormalities, which are dose-dependent (Axelsson 1977; Gottschalk et al., 1978; Llerena et al., 2002). Heiman (1977) reported cases of life-threatening ventricular arrhythmia in patients who had ingested a combination of thioridazine and imipramine or amitriptyline, alerting clinicians to the risk of using thioridazine at high doses and in combinations with tricyclic antidepressants. Therefore, knowledge of the thioridazine metabolism seems to be of importance.

Like other phenothiazine neuroleptics, thioridazine undergoes S-oxidation in the thiazine ring in position 5, as well as aromatic hydroxylation (mainly in position 7), N-demethylation, and N-oxidation (Papadopoulos et al., 1985; Svendsen and Bird, 1986; Lin et al., 1993). However, unlike other phenothiazines, thioridazine forms a sulfoxide in position 2 of the thiomethyl substituent (mesoridazine), which is further oxidized to a sulfone (sulforidazine) (Fig. 1). Metabolites formed by S-oxidation in position 2, i.e., mesoridazine and sulforidazine, are more potent than thioridazine in blocking dopaminergic D2 and noradrenergic α1 receptor-blocking properties of monoo- and di-sulfones, the obtained results are of pharmacological and clinical importance, in particular, in a combined therapy. Knowledge of the catalysis of thioridazine metabolism helps to choose optimum conditions (a proper coadministered drug and dosage) to avoid undesirable drug interactions.

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ABBREVIATIONS: P450, cytochrome P450; DDC, diethylidithiocarbamic acid; HPLC, high performance liquid chromatography; K_m, the Michaelis constant; V_max, maximum velocity of the reaction.
Thus, there are no complete data on the enzymatic catalysis of thioridazine metabolism in humans. Many other P450 enzymes that might be involved in the mono-2-sulfoxidation of thioridazine, as well as the catalysis of other metabolic pathways of this neuroleptics, such as di-2-sulfoxidation, N-demethylation, and 5-sulfonation processes, have not been studied in humans so far. Considering the serious side effects of thioridazine and the risk of its application in a combination therapy, in the present study, we aimed to concurrently investigate the contribution of human P450s to thioridazine mono-2-, di-2-, and 5-sulfoxidation, and N-demethylation using different in vitro models.

The obtained results indicate that CYP1A2 and CYP3A4 are the main enzymes responsible for 5-sulfoxidation and N-demethylation, whereas CYP2D6 is the key enzyme that catalyzes mono-2- and di-2-sulfoxidation of thioridazine in human liver; besides CYP2D6, CYP3A4 significantly contributes to thioridazine mono-2-sulfoxidation. The above results are compared with the findings of analogous experiments with other phenothiazine neuroleptics and are discussed with respect to the substrate-structure differences in the enzymatic catalysis of the metabolism of phenothiazines. Moreover, pharmacological and clinical aspects of the obtained results are emphasized.

Materials and Methods

Drugs and Chemicals. Thioridazine hydrochloride was obtained from Jelfa (Jelenia Góra, Poland). Mesoridazine and sulforidazine (free bases) were donated by Sandoz Pharma AG (Basel, Switzerland). Thioridazine 5-sulfoxide and N-desmethylthioridazine were synthesized in our laboratory as described previously (Daniel et al., 1997). α-Naphthoflavone, diethylthiocarbamic acid (DDC), quinidine, sulfaphenazole, ticlopidine, ketoconazole, and NADPH were purchased from Sigma (St. Louis, MO). All the organic solvents with HPLC purity were supplied by Merck (Darmstadt, Germany).

Human Liver Microsomes. Human liver microsome preparations (HK23, HK25, HK37, HG03, HG43, HG56, HG89, HG93) were obtained from BD Gentest (Woburn, MA).

Liver microsomes from patients HK23, HG43, and HG89 were used for optimizing the conditions of thioridazine metabolism. Microsomal protein, 250 μg, was resuspended in 500 μl of 20 mM Tris/HCl buffer (pH 7.4). To determine enzyme kinetic parameters, the thioridazine concentrations used ranged from 5 to 250 μM. For studies of thioridazine metabolism in individual patients, 25 μM thioridazine was used. For inhibition studies, 25 μM thioridazine was incubated with the selective P450 inhibitors: 2 μM α-naphthoflavone (a CYP1A2 inhibitor), 200 μM DDC (a CYP2A6 inhibitor), 10 μM sulfaphenazole (a CYP2C9 inhibitor), 10 μM quinidine (a CYP2D6 inhibitor), and 2 μM ketoconazole (a CYP3A4 inhibitor). After a 3-min preincubation at 37°C, the reaction was initiated by adding NADPH to a final concentration of 1 mM. After a 25-min incubation, the reaction was stopped by adding 200 μl of methanol. Thioridazine and its metabolites were analyzed by an HPLC method as described below.

Correlation Analysis of the Data. The rates of thioridazine mono-2-, di-2-, and 5-sulfonation, and N-demethylation were correlated with the rates of P450-specific reactions: phenacetin O-deethylation (CYP1A2), coumarin 7-hydroxylation (CYP2A6), S-mephenytoin N-demethylation (CYP2B6), diclofenac 4′-hydroxylation (CYP2C9), S-mephenytoin 4′-hydroxylation (CYP2C19), bufuralol 1′-hydroxylation (CYP2D6), chlorzoxazone 6-hydroxylation (CYP2E1), and testosterone 6β-hydroxylation. Each data pair was
compared by a simple linear regression analysis using the statistical program
Prism 2.01 (GraphPad Software Inc., San Diego, CA). Monoxygenase activities
for each liver microsomal preparation (donor) were determined and
provided by BD Gentest.

dDNA-Expressed Human P450s. Microsomes from baculovirus-infected
insect cells expressing CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19,
CYP2D6, CYP2E1 and CYP3A4 (Supersomes) were obtained from Gentest
Co. (Woburn, MA., USA). Thoridazine metabolism was studied under exper-
imental conditions similar to those described for liver microsomes, with 10, 25,
50, 100 and 250 μM thioridazine, except for the fact that the final concentration
of P450s was 100 pmol/ml. Thoridazine and its metabolites were analyzed by
HPLC as described below.

Determination of Thoridazine and Its Metabolites in the Incubation
Medium. Thoridazine and its metabolites were quantified using the previ-
ously described HPLC method (Daniel et al., 1997). After incubation, the
samples were centrifuged for 10 min at 2000 g. The water phase containing
thioridazine and its metabolites was extracted (pH 12) with hexane and 1.5%
isoamyl alcohol. The residue obtained after evaporation of the microsomal
extracts was dissolved in 100 μl of the mobile phase described below. An
aliquot of 20 μl was injected into the HPLC system. The concentrations of
thioridazine and its metabolites (mesoridazine, sulforidazine, thioridazine
5-sulfoxide, N-desmethylothioridazine) were assayed using a LaChrom (Merck-
Hitachi) HPLC system with fluorescence detector. The analytical column
(Econosphere C18, 5 μm, 4.6 × 250 mm) was purchased from Alltech
(Carnforth, UK). The mobile phase consisted of an acetate buffer, pH 3.4
(100 mmol of ammonium acetate, 20 mmol of citric acid, and 1 ml of
triethylamine in 1000 ml of the buffer, adjusted to pH = 3.4 with an 85%
phosphoric acid), and acetonitrile in the proportion 50:50. The flow rate was
1.5 ml min⁻¹; the column temperature was ambient. The fluorescence was
measured at a wavelength of 270 nm (excitation) and 467 nm (emission) for
thioridazine, mesoridazine, sulforidazine, and N-desmethylothioridazine, and
274/388 nm for thioridazine 5-sulfoxide. The compounds were eluted in the
following order: sulforidazine (5.49 min), mesoridazine (7.82 min), diastereoi-
ders of thioridazine 5-sulfoxide (9.06 min and 10.27 min), N-desmethylthy-
oridazine (12.86 min), and thioridazine (16.81 min). The sensitivity of the
HPLC method allowed us to quantify thioridazine as low as 0.004 nmol/ml,
mesoridazine and thioridazine 5-sulfoxide as low as 0.002 nmol/ml, and
sulforidazine and N-desmethylothioridazine as low as 0.001 nmol/ml microso-
mal suspension. The reproducibility coefficient of variation was under 3%.

Results

Thioridazine Metabolism in Human Liver Microsomes. Figure
2, A to D, shows the representative Eadie-Hofstee plots for thiorida-
zine oxidation processes in liver microsomes from the patient HK23.
All the plots were nonlinear, except for the plot for thioridazine
di-2-sulfoxidation. Similar results were obtained with liver micro-
somes from patients HG43 and HG89.

Correlation Study. Interindividual variability of the rates of thio-
idazine metabolism ranged from 2.5-fold (N-demethylation) to 3.9-
fold (5-sulfoxidation) (Fig. 3).

The rates of formation of thioridazine metabolites by different
preparations of human liver microsomes (Fig. 3) were compared with
the monoxygenase activities determined for each liver preparation by
the supplier (BD Gentest). The results of those analyses are shown in
Table 1, where the correlation coefficient (r) and the
p value are given for each pair of data. The formation of thioridazine 5-sulfoxide
and N-desmethylthioridazine was significantly correlated with phenacetin
A correlation (r value) between the rate of thioridazine metabolism and the velocity of P450-specific reactions in human liver microsomes

Each pair of data was compared by a simple linear regression analysis using the statistical program Prism 2.01 (GraphPad Software, Inc.). Statistical significance was indicated with ** p < 0.01 and * p < 0.05; N.S. (p > 0.05) is statistically not significant.

O-deethylation (CYP1A2) and testosterone 6β-hydroxylase (CYP3A4) activities, whereas the production of thioridazine mono-2-sulfoxide (mesoridazine) and thioridazine di-2-sulfoxide (sulforidazine) was correlated well with bufuralol 1’-hydroxylation activity (CYP2D6). Moreover, thioridazine mono-2-sulfoxidation also correlated significantly with testosterone 6β-hydroxylase activity (CYP3A4).

No correlation was observed between the production of thioridazine metabolites and the rates of coumarin 7-hydroxylation (CYP2A6), S-mephenytoin N-demethylation (CYP2B6), dicyfenac 4’-hydroxylation (CYP2C9), S-mephenytoin 4’-hydroxylation (CYP2C19), and chlorozoxazone 6-hydroxylation (CYP2E1).

Inhibition of Thioridazine Metabolism by P450-Selective Inhibitors. α-Naphthoflavone (a CYP1A2 inhibitor) and ketoconazole (a CYP3A4 inhibitor) significantly decreased the rate of thioridazine 5-sulfoxidation (up to 52 and 62% of the control value, respectively) and thioridazine N-demethylation (up to 54 and 48% of the control value, respectively) (Fig. 4). On the other hand, quinidine (a CYP2D6 inhibitor) exerted a strong inhibitory effect on the rate of thioridazine mono-2- and di-2-sulfoxidation (up to 48 and 35% of the control value, respectively) (Fig. 4). Moreover, ketoconazole significantly reduced the rate of thioridazine mono-2-sulfoxidation (up to 76% of the control value). DDC (a CYP2A6 + CYP2E1 inhibitor), sulfaphenazole (a CYP2C9 inhibitor), and ticlopidine (a CYP2C19 inhibitor) had no inhibitory effect on the rate of thioridazine metabolism.

Study with cDNA-Expressed Human P450s. The Lineweaver-Burk analysis of the thioridazine metabolism in cDNA-expressed human P450s is presented in Table 2. The obtained kinetic parameters showed distinct inter-isoform differences, which was consistent with the multienzyme Eadie-Hofstee plots derived from liver microsomes (Fig. 2, A–D). The highest intrinsic clearance (V_max/K_m) was found for CYP1A2 and CYP3A4 in the case of sulfuridazine and N-demethylation, and for CYP2D6 in the case of mono-2- and di-2-sulfoxidation.

The ability of cDNA-expressed human P450s to metabolize thioridazine at its low, therapeutic concentration (10 μM) is shown in Fig. 5, A to D. According to the calculated intrinsic clearance values, the preference of P450 isoforms for catalyzing thioridazine metabolism was as follows (pmol of product/μmol of P450 isofrom/min): 2D6 > 2C19 > 1A2 ≈ 2B6 > 3A4 > 2C9 for mono-2-sulfoxidation; 2D6 > 2C19 > 1A2 > 3A4 for di-2-sulfoxidation; 1A2 > 3A4 > 2E1 ≈ 2A6 > 2B6 > 2D6 > 2C9 > 2C19 for 5-sulfoxidation; and 1A2 > 2C19 > 3A4 > 2D6 for N-demethylation. CYP2A6 and CYP2E1 did not produce mesoridazine at a measurable amount, whereas sulfuridazine and N-desmethylthioridazine were not formed by CYP2A6, CYP2B6, CYP2C9, and CYP2E1.

Quantitative Estimation of the Contribution of P450 Enzymes to the Particular Metabolic Pathways of Thioridazine. We roughly

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**TABLE 1**

<table>
<thead>
<tr>
<th>Phenacetin</th>
<th>Coumarin</th>
<th>S-Mephenytoin</th>
<th>Dicyfenac</th>
<th>Bufuralol</th>
<th>Chlorozoxazone</th>
<th>Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O-Deethylation (CYP1A2)</td>
<td>2%</td>
<td>5%</td>
<td>0%</td>
<td>10%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>7-Hydroxylation (CYP2A6)</td>
<td>10%</td>
<td>20%</td>
<td>30%</td>
<td>40%</td>
<td>50%</td>
<td>60%</td>
</tr>
<tr>
<td>S-Mephenytoin N-Demethylation (CYP2B6)</td>
<td>10%</td>
<td>20%</td>
<td>30%</td>
<td>40%</td>
<td>50%</td>
<td>60%</td>
</tr>
<tr>
<td>4’-Hydroxylation (CYP2C9)</td>
<td>10%</td>
<td>20%</td>
<td>30%</td>
<td>40%</td>
<td>50%</td>
<td>60%</td>
</tr>
<tr>
<td>5-Sulfoxidation (CYP2C19)</td>
<td>10%</td>
<td>20%</td>
<td>30%</td>
<td>40%</td>
<td>50%</td>
<td>60%</td>
</tr>
<tr>
<td>1’-Hydroxylation (CYP2D6)</td>
<td>10%</td>
<td>20%</td>
<td>30%</td>
<td>40%</td>
<td>50%</td>
<td>60%</td>
</tr>
<tr>
<td>6β-Hydroxylation (CYP3A4)</td>
<td>10%</td>
<td>20%</td>
<td>30%</td>
<td>40%</td>
<td>50%</td>
<td>60%</td>
</tr>
</tbody>
</table>

**TABLE 2**

Kinetic parameters of thioridazine metabolism in cDNA-expressed human P450s (Supersomes)

<table>
<thead>
<tr>
<th>P450</th>
<th>Thioridazine Mono-2-sulfoxidation</th>
<th>Thioridazine Di-2-sulfoxidation</th>
<th>Thioridazine 5-Sulfoxidation</th>
<th>Thioridazine N-Demethylation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V_max (μM)</td>
<td>V_max/K_m (μM)</td>
<td>V_max (μM)</td>
<td>V_max/K_m (μM)</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>160</td>
<td>1.786</td>
<td>0.0112</td>
<td>16</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>323</td>
<td>0.485</td>
<td>0.0015</td>
<td>48</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>67</td>
<td>0.605</td>
<td>0.0090</td>
<td>55</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>45</td>
<td>0.221</td>
<td>0.0049</td>
<td>91</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>47</td>
<td>6.289</td>
<td>0.1338</td>
<td>26</td>
</tr>
<tr>
<td>CYP2DC</td>
<td>62</td>
<td>16.667</td>
<td>0.2688</td>
<td>10</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>333</td>
<td>0.527</td>
<td>0.0016</td>
<td>38</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>42</td>
<td>0.302</td>
<td>0.0072</td>
<td>20</td>
</tr>
</tbody>
</table>

The highest intrinsic clearances are shown in bold.
estimated the contribution of the P450 enzymes studied here to thioridazine mono-2-, di-2-, and 5-sulfoxidation, and N-demethylation in liver microsomes on the basis of the rate of those reactions in the Supersomes and the contribution of each isoform to the total P450 content in the human liver. Calculations performed at a low (therapeutic) concentration of thioridazine (10 μM) indicate that CYP2D6 is the main isoform responsible for mono-2- and di-2-sulfoxidation (48.6 and 63.6%, respectively), whereas CYP1A2 and CYP3A4 are the key enzymes that catalyze 5-sulfoxidation (46.5 and 34.3%, respectively) and N-demethylation (44.1 and 51.6%, respectively) of thioridazine in the human liver (Table 3). CYP3A4 contributes to a lesser degree to thioridazine mono-2-sulfoxidation (21.7%) (Table 3). The results obtained at 10 μM and 100 μM (data not shown) thioridazine were similar.

In the liver, the amount of a metabolite formed by an individual P450 enzyme depends on both its catalytic activity with respect to the product formation and its relative contribution to the total P450 content. Therefore, although the intrinsic clearance found for CYP2C19 was higher than that for CYP3A4 regarding mono-2-sulfoxidation (Table 2), the calculated contribution of CYP3A4 to this reaction was considerably greater than that of CYP2C19 (Table 3), as a result of their relative amounts in the total liver P450 (30% and 1%, respectively).

Discussion

The results presented above indicate a major contribution of CYP1A2 and CYP3A4 to thioridazine 5-sulfoxidation and N-demethylation, and of CYP2D6 to its mono-2- and di-2-sulfoxidation. It is also worth stressing that CYP3A4 contributes to mono-2-sulfoxidation of thioridazine. The above final conclusion is based on our consistent results of the Eadie-Hofstee analysis (nonlinear plots suggesting multiple-enzyme catalysis), correlation and inhibition studies, and demonstration of the ability of cDNA-expressed P450s to metabolize thioridazine. It should be mentioned that the latter results (based on the average values of P450s in the liver) are theoretical, since actual results would depend on the interindividual variability of P450s and the diverse contribution of individual P450s to the total content of P450 protein in the liver.

The obtained results are similar to our recent data (Wójcikowski et al., 2003, 2004) showing the main contribution of CYP1A2 and CYP3A4 to thioridazine metabolism by P450 enzymes.

### Table 3

<table>
<thead>
<tr>
<th>P450</th>
<th>Relative Contribution of the Isoform to the total P450 Contents in Liver Microsomes (Fraction)*</th>
<th>Relative Contribution of the Isoform to Thioridazine Metabolism in Liver Microsomes (Percentage)$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>0.127$^a$</td>
<td>Thioridazine Mono-2-sulfoxidation 10.8 Thioridazine Di-2-sulfoxidation 16.7 Thioridazine 5-Sulfoxidation 46.5 Thioridazine N-Demethylation 44.1</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>0.040$^a$</td>
<td>N.D.</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>0.002$^a$</td>
<td>0.2 N.D.</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>0.180$^a$</td>
<td>6.6 N.D.</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>0.010$^a$</td>
<td>12.1 6.1 N.D.</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>0.015$^a$</td>
<td>48.6 63.6 0.9 1.6</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>0.066$^a$</td>
<td>N.D. 6.0 N.D.</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>0.288$^a$</td>
<td>21.7 13.6 34.3 56.1</td>
</tr>
</tbody>
</table>

N.D., not detected.

* Data according to $^a$ Shimada et al. (1994) and $^b$ Lewis (2001).

$^+$ Relative contribution of P450s to the particular metabolic pathways of thioridazine was calculated as percentage of the sum of predicted velocities in liver microsomes. The predicted velocity in liver microsomes was calculated by multiplying the velocity in Supersomes (see Fig. 5) by the relative contribution of isoform to the total P450 content in liver microsomes. For details, see Wójcikowski et al. (2003, 2004).

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**FIG. 5.** Biotransformation of thioridazine via mono-2-sulfoxidation (A), di-2-sulfoxidation (B), 5-sulfoxidation (C), and N-demethylation (D) by the cDNA-expressed human P450s (Supersomes). Thioridazine (10 μM) was incubated with Supersomes (100 pmol P450/ml) and NADPH (1 mM) for 25 min. Each bar represents the mean value ± S.D. of three determinations from independent incubations.
the 5-sulfoxidation (in the thiazine ring) of the aliphatic-type phenothiazine neuroleptic promazine (31 and 39%, respectively) and the piperazine-type neuroleptic perazine (32 and 30%, respectively). As for promazine and perazine, the 5-sulfoxidation of thioridazine (also in the thiazine ring) was mediated by CYP1A2 (46%) and CYP3A4 (34%). However, some interdrug differences were observed in the catalysis of side chain N-demethylation. The contribution of CYP1A2 and CYP2C19 to promazine N-demethylation was similar (35 and 32%, respectively), whereas CYP2C19 was the main isoenzyme catalyzing perazine N-demethylation (68%). On the contrary, CYP2C19 contributed only marginally to thioridazine N-demethylation (4%). This metabolic pathway of thioridazine was mediated by CYP1A2 (44%) and CYP3A4 (52%).

The above discrepancy may stem from different chemical structures of the side chains of the phenothiazines studied (thioridazine-piperidinidine side chain, promazine-aliphatic side chain, perazine-piperazine side chain), which influence their access to and the interaction with catalytic sites of cytochrome P450. The minor contribution of CYP2C19 to thioridazine N-demethylation in a side chain might be due to strict structural requirements of the enzyme for its substrates and to limited space around the nitrogen in the side chain (compared with promazine and perazine), which hinders oxidation of the chemically vulnerable position by CYP2C19.

The results obtained in our experiment agree with clinical studies demonstrating that CYP2D6 is the key enzyme catalyzing thioridazine 2-sulfoxidation (Meyer et al., 1990; Llerena et al., 2000, 2001; Berezcz et al., 2003) and that CYP1A2, in addition to CYP2D6, is involved in the metabolism of thioridazine (Berezcz et al., 2003). However, our calculated data showed that besides CYP2D6 (49%), CYP3A4 (22%) was substantially engaged in mono-2-sulfoxidation of thioridazine. Thus, considering the higher contribution of CYP2D6 to di-2-sulfoxidation (64%) than to mono-2-sulfoxidation (49%) of thioridazine, it seems that the sulforidazine/mesoridazine ratio might be an additional and more specific marker of CYP2D6 activity than the mesoridazine/thioridazine ratio, proposed by Llerena et al. (2000). The above suggestion is supported by the studies of von Bahr et al. (1991), which showed that the plasma concentration of sulforidazine was influenced by the debrisoquine hydroxylation phenotype more than was the mesoridazine level in humans. Moreover, considering an easily detectable concentration of sulforidazine in human plasma (although one half to one quarter of the concentration of mesoridazine) (Cohen et al., 1989; Meyer et al., 1990), the simultaneous measurement of both metabolites in plasma and determination of the two markers of CYP2D6 activity is possible. It is also important to note that in light of our results, the CYP2D6 activity indicator expressed as the sulforidazine/mesoridazine ratio, proposed by Llerena et al. (2000), might be an additional and more specific marker of CYP2D6 activity than the mesoridazine/thioridazine ratio.

In summary, the results of the present study show that 1) different structures of phenothiazine neuroleptics (mainly the structure of a side chain) influence their interactions with catalytic sites of cytochrome P450; 2) CYP1A2 and CYP3A4 are the main enzymes responsible for 5-sulfoxidation and N-demethylation, whereas CYP2D6 is the basic enzyme that catalyzes mono-2- and di-2-sulfoxidation of thioridazine in human liver; 3) besides CYP2D6, CYP3A4 significantly contributes to thioridazine mono-2-sulfoxidation; 4) the relative contribution of CYP2C19 to thioridazine N-demethylation is marginal; and 5) the sulforidazine/mesoridazine ratio might be an additional and more specific marker of CYP2D6 activity than the mesoridazine/thioridazine ratio.

References

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